MICROBIOLOGY

A. BACTERIOLOGY: Routine bacteriology services are available. Virology, mycology, parasitology, and TB testing are sent to Reference Labs.

1. General collection and ordering information

a. All specimens must be adequately labeled, and if not ordered in CHCS, accompanied by a properly completed microbiology request including: patient name, name of physician, location collected, **source** of the culture, type of test requested, date and time of specimen collection.

b. Rejection criteria:

(1) Unlabeled specimen
(2) Dried swab
(3) Improperly collected or inadequate sample
(4) Improper transportation
(5) Improper storage
(6) Leaking sample container
(7) Gross external contamination of sample
(8) Frozen, dehydrated or outdated media

c. The Microbiology Laboratory should be alerted in the event any specimen is suspected of containing especially hazardous organisms such as tularemia, Brucella, plague, AFB, etc. This information should be communicated to the laboratory in the test order.

d. If routine cultures fail to provide a microbial diagnosis, consultation with the laboratory is suggested and specialized handling and procedures may be authorized. This will prevent further samples from being processed in the same manner that failed initially.

e. If unusual or fastidious organisms are sought, the laboratory should be so advised on the requisition in order that special handling and appropriate media may be used (Example: nasopharyngeal culture for Neisseria meningitidis/gonorrhoeae or Bordetella pertussis). **NOTE**: Bordetella pertussis requires a 24-hour notice in advance to arrange for transportation to UMC.

diagnosis, or pathogens are suspected. Unless so noted, only routine laboratory methods will be used. Routine methods often fail to detect unusual pathogens. The physician's failure to supply the above information may produce misleading data.

2. Collection Materials:

   a. Routine specimen collection materials, such as sterile needles and syringes, transport tubes, swabs, cups, etc., are available from central supply.

   b. The following materials are only available from the microbiology laboratory:

      (1) Stool culture transport media (Enteric pathogen transport)
      (2) GC culture medium: For the collection and transport of specimens for Neisseria gonorrhoeae. (Martin-Lewis pill pocket plates).
      (3) Blood Culture Bottles for adult and pediatric draws.

3. Abscesses, Exudates, Transudates, and Wounds:

   a. Specimen Collection:

      (1) Aspirates: These should be drawn in a sterile syringe. Cleanse the area with 70% alcohol, 2% iodine or providone iodine preparation and allow to sit for approximately one minute. Aspirated material may be submitted in the syringe, WITHOUT the needle; cap with a sterile cap prior to transport.
      (2) Swabs: If collection must be made with a sterile swab, care must be taken to avoid contamination with surrounding microbial flora. Purulent material may be exuded from lesions after cleansing of the surrounding area. Please submit a separate swab or smear for Gram stain purposes. Transport immediately.

4. Anaerobic Cultures:

   a. Specimen Collection: Avoid contamination of specimens with normal flora (e.g., mouth, skin, vagina or bowel).

   b. Special anaerobic collection and transport swabs are available from central supply.

e. Hold and transport specimens at ambient temperature; NEVER REFRIGERATE.

f. Syringe and needle aspirates submitted in the syringe must be processed within 30 minutes of collection.

5. Blood Cultures:

   a. Specimen Collection: Bottles available are adult aerobic and anaerobic bottles that require 5-10 mls of blood each, and pediatric aerobic bottles that require 1-4 mls of blood. **NOTE**: Bottles are not self limiting – observe when correct amount of blood has been drawn and remove bottle. Keep bottles upright while drawing.

   b. Clean site with 70% alcohol then 10% iodine. Clean top of bottle with 10% iodine and allow to dry. Do not remove.

6. Cebrospinal Fluid:

   a. Specimen Collection: Cerebrospinal fluid is usually collected in three sterile screw cap tubes found in spinal sets. Collect a minimum of 3 ml in each tube and label the tubes 1,2, and 3 in order of collection. Submit tube 1 to Chemistry, tube 2 to Microbiology, and tube 3 to Hematology. Bring to the laboratory immediately.

7. Eye cultures:

   a. Specimen collection: Eye cultures require close coordination between the laboratory and the physician. Transport immediately. If Neisseria gonorrhoeae is suspected, the physician must plate the specimen to Martin Lewis agar, pill placed in well, plate placed in plastic ziplock bag, and delivered to laboratory. Place collected swab in transport tube, squeeze to dampen swab with media.

8. Nasal, Nasopharyngeal, and Sputum Cultures:

   a. Specimen collection: Nasal cultures are usually obtained with a swab. Nasopharyngeal swabs for Bordatella pertussis are collected on Nicrome wire swabs. RSV's require nasopharyngeal washes. After collection of the specimen, insert the swab into the collection tube and break the capsule of

9. Smears:

    a. GC (gonorrhea) smears: Microscopic demonstration of gram negative, intra-cellular diplococci in smears of urethral exudate from MEN constitutes sufficient basis for a diagnosis of gonorrhea. Smears are **not** exclusively diagnostic for the diagnosis of gonorrhea in WOMEN.

    b. Gram stain: The gram stain is a vital part of the initial steps used to identify bacteria. Gram stains are automatically performed on all fluid and tissue specimens. A separate swab or smear is recommended. Gram stains are not performed on feces or urine specimens.

10. Sterility and Environmental Cultures: Cultures for sterility and environmental assay are only processed after consultation with the Microbiology Section. Positive cultures are reported in accordance with established protocols governing the type of culture processed.

11. Stool Cultures:

    a. Specimen collection: Stool for culture should be placed immediately into a yellow or orange C&S collection vial. Pediatric stool on infants can be collected on a sterile swab and delivered to the laboratory immediately. Pediatric stools on children in diapers can be collected over time as long as sample is placed immediately into C&S collection vial until proper amount is collected.

    b. Stools are routinely screened for Salmonella, Shigella, E.coli 0157 and Campylobacter. If Yersinia is suspected, annotate in CHCS under order comment.

12. Throat Cultures:

    a. Specimen collection: The sterile swab should be inserted in a manner such that contamination with oral flora is avoided. Swab areas of exudate or inflammation. Rub tonsils vigorously. DO NOT touch tongue or side of mouth.

    b. Routine throat swabs are primarily screened for the presence of beta-hemolytic streptococci.

14. GC Cultures:

    a. Specimen collection: The Martin Lewis Pill Pocket plate is used for the collection and transport of GC cultures. Allow the Martin Lewis plate to come to room temperature before inoculation. Roll the swab that contains the specimen directly on the medium in a "Z" pattern to assure adequate exposure of the swab to the medium.. Place the CO_2 generator pill in the well, place the inoculated plate inside a transport bag and close bag. No water is needed. Send to the laboratory ASAP.

15. Chlamydia collection:

    a. Follow directions on Chlamydia collection kits, which are obtained from Medical Supply. Collect specimen for GC cultures prior to Chlamydia collection.

    b. Chlamydia test method is the genprobe method. All Chlamydia tests automatically receive a GC genprobe. If turnaround time is not an issue, do not order a separate GC culture.

16. Tuberculosis Cultures: TB cultures are sent to a reference lab. Call lab for more information.

17. Urine Culture:

    a. First morning midstream collections are preferred. Urine must be transported and processed within one hour of collection. If delay is anticipated, refrigerate 2-6^{o} C.

    b. Urine cultures are performed by request of the physician and when routine urinalysis indicates a positive nitrite or leukocyte esterase test.

18. Vaginal Cultures:

    a. Vaginal Gram stains are not routinely performed in the microbiology laboratory. Cultures from the vagina should be ordered as Genital cultures and clearly labeled (in the Comments field in CHCS to indicate the clinical problem, i.e. vaginosis or if looking for yeast. Collect with swab, transport media collection system.

B. ANTIBIOTIC SUSCEPTIBILITY TESTING

1. VITEK Automated Sensitivity Reporting is the primary method. These are pre-made cards for gram negative and gram positive organisms as required by NCCLS. Sensitivity cards are coordinated closely with pharmacy formulary.

2. Policies:

   a. Antimicrobial susceptibility testing is not routinely performed on the following isolates:

      Anaerobes
      Haemophilus sp. – Beta Lactamase is performed
      Beta Streptococcus Group A
      Neisseria sp. except for N. meningitidis

   b. Susceptibility testing will always be performed on all isolates from blood or CSF.

   c. All N. gonorrhoeae are cultured on Thayer Martin or Martin Lewis agar and tested for beta-lactamase production.

   d. Susceptibility testing on all organisms will be performed for the following antibiotics:

Staphs	Streps*	Gram Negatives
Ampicillin	Ampicillin	Amoxicillin/Clavulanic Acid
Ampicillin/sulbactam	Cefazolin	Ampicillin
Cefazolin	Ciprofloxacin	Cefazolin
Ciprofloxacin	Clindamycin	Ceftriaxone
Clindamycin	Erythromycin	Ciprofloxacin
Erythromycin	Levofloxacin	Gentamicin
Gentamicin	Nitrofurantoin	Levofloxacin
Oxacillin	Penicillin-G	Minocycline
Penicillin-G	Tetracycline	Tobramycin
Rifampin	Tobramycin	Trimeth-Sulfa
Tetracycline	Trimeth-Sulfa	
Trimeth-Sulfa	Vancomycin	

C. PARASITOLOGY:

1. Specimen Collection: Each ward/clinic must order O&P collection kits through medical supply.

   a. Feces: Three normally passed stools, collected over a 2 - 3 day span, are the preferred specimens. Specimens may be collected directly in plastic cups or from clean dry sheets of paper; immediately transfer feces into O&P collection vial containing preservative. Specimens MUST NOT be contaminated with urine or collected from toilet water. The demonstration and identification of intestinal parasites is greatly inhibited if feces are collected during times when the patient is taking antacids, antidiarrheal compounds, antibiotics, mineral oils, or compounds containing barium, bismuth, or magnesia. Specimens should be transported at ambient temperatures but protected against extreme changes in temperature. DO NOT use artificial means to keep the specimen at body temperature. After collection, fill each vial to the full line on each vial and mix well. Submit to the laboratory for shipping. No diapers accepted by lab.

   b. Blood Parasites: Slides will be sent to a consulting pathologist for confirmation

      (1) Malaria: Thin and thick blood films are ideally prepared just prior to fever spikes, after fever patterns have been established. If fever patterns have not been established, several random samples may be required.
      (2) Filariasis: Some authors recommend that thin and thick films are best prepared between 2200 and 0200 hours, due to the nocturnal migration of Wuchereria bancrofti. Direct wet mounts of peripheral blood may also be helpful.
      (3) Leishmaniasis: Leishmanial forms are found only rarely in peripheral blood.
      (4) Trypanosomiasis: Thick and thin peripheral blood films, direct blood wet mounts, CSF, and lymph node exudates may all be helpful for the laboratory diagnosis of trypanosomiasis.

D. MYCOBACTERIOLOGY (TB):

1. Acid Fast (TB) Laboratory: All TB requests are sent to Reference Laboratories. Specimens must be collected in clean, shipping tubes obtained from the laboratory. The specimen container must bear the patient's name, SSN, location of the specimen, and physician's name. Mycobacteriology (TB) culture procedures require lengthy time frames. Culture results will not be available for several weeks (eight

3. Urine: A series of daily, single, mid stream urines, voided early in the morning, should be submitted. Twenty four-hour collections will not be accepted.

4. Tissue: Tissue to be processed for AFB should be collected aseptically and kept moist with sterile, non bacteriostatic physiologic saline, and transported to the laboratory as soon as possible.

5. Other Specimens: Culture of all other locations must follow consultation with the microbiology supervisor. These may include any aseptically collected specimens. Body fluids should be kept from clotting with a small amount of heparin. Swabs may be acceptable when a more voluminous specimen cannot be obtained. Ordinarily, blood and stool will not be processed for AFB.

E. MYCOLOGY (FUNGI): All mycology culture requests are sent to Reference Laboratories with the exception of KOH wet preps.

F. VIROLOGY:

1. All Virology specimens are sent to Reference Laboratories.

2. General Principles:

a. Specimens generally should be collected within 48 hours of onset of symptoms.

b. Request forms should bear complete information including date and time of collection, specimen source, symptomology, etc. Whenever possible, the viruses considered the most likely agents should be indicated. This allows the selection of proper techniques, cell lines for inoculation, etc. Specimens labeled "viral studies" are not acceptable. The physician must specify which virus(s) to culture.

c. Specimens should be submitted in appropriate transport media available from the laboratory. Thaw immediately before use. Specimens should be hand carried to the hospital laboratory, where they will be refrigerated, and sent out as soon as possible.

d. Serologic testing should be carried out concurrently with viral isolation attempts. The acute serum sample should be drawn at the time of specimen collection. Generally, acute and convalescent sera should be processed together.